

# GENETIC DIVERGENCE WITHIN THE GENUS *LIRIODENDRON* (MAGNOLIACEAE)<sup>1,2</sup>

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## ABSTRACT

The genus *Liriodendron* L. consists of a southeast Asian–eastern North American disjunct species pair, but the genus had a much wider distribution in the Northern Hemisphere during the late Cretaceous and the Tertiary. Although generally similar in morphology, the two extant species are measurably different. In 1973 and 1977 they were hybridized, and interspecific heterosis was observed in the progeny. After seven years, the interspecific hybrids synthesized in 1973 had a significantly greater biomass than intraspecific hybrids of *L. tulipifera*. The growth rate of interspecific and intraspecific F<sub>2</sub> seedlings was approximately equal. A preliminary survey of flavonoid extracts in *L. tulipifera* populations showed a high level of quantitative variation that was not obviously correlated with either geographic or environmental parameters. While there was little flavonoid variation in one small *L. chinense* population, a tree from a second locality in China was markedly divergent in its chromatographic pattern. An isozyme survey of individual trees from a few populations showed *L. tulipifera* to be moderately heterozygous. Six trees of *L. chinense* from one locality were identically homozygous, while a seventh tree from a different locality was equally homozygous but for different alleles at a number of loci. The available evidence indicates that the two *Liriodendron* species have not diverged very far from a presumed common ancestor. However, *L. tulipifera* is an abundant, almost weedy, species in some parts of its range, whereas *L. chinense* has suffered from population restriction and loss of heterozygosity to the degree that it shows inbreeding depression.

The genus *Liriodendron* includes two extant species, *L. chinense* (Hemsl.) Sarg. and *L. tulipifera* L., and a number of extinct species that are based on fossils of leaves or seeds from various Cretaceous and Tertiary strata. The Chinese tulip tree occurs in widely scattered populations from the northern part of Indochina to the eastern Chinese provinces of Anhwei and Hupeh (Fig. 1). From conversations with botanists from the People's Republic of China, it appears that *L. chinense* exists as a small number of populations each containing only a few individuals. Unlike the Chinese species, *L. tulipifera* is abundant and often weedy over much of its range, which includes nearly all of the United States east of the Mississippi River (Fig. 1).

Fossils in deposits of Tertiary age from Asia, Europe, and North America indicate that *Liriodendron* was once much more widely distributed in the Northern Hemisphere. It survived in Europe at least until the Pliocene and early Pleistocene (Szafer, 1954), and is represented in west-

ern North American Paleogene (Wolfe, 1972) and Neogene sediments (Smiley & Rember, 1981). Considering this paleobotanical evidence, it is probable that *Liriodendron* was widespread in the Northern Hemisphere in what has been termed the Arcto-Tertiary Forest (Chaney, 1959), and that its present distribution reflects range restrictions due to local extinction at various times in the late Cenozoic. There is no evidence that populations of contemporary *L. tulipifera* and *L. chinense* were ever sympatric, although these taxa may survive as relicts of antecedents whose distribution was originally sympatric.

Despite the long isolation, the two extant species are highly interfertile (He & Santamour, pers. comm.; Santamour, 1972; Miller & Parks, 1980). In 1973 two of us (Miller & Parks) set up a long-range hybridization study to explore the genetic behavior of the F<sub>1</sub> hybrid and advanced generations derived from it. This work is being augmented by analyses of inter- and intraspecific variability as determined from morphology, bio-

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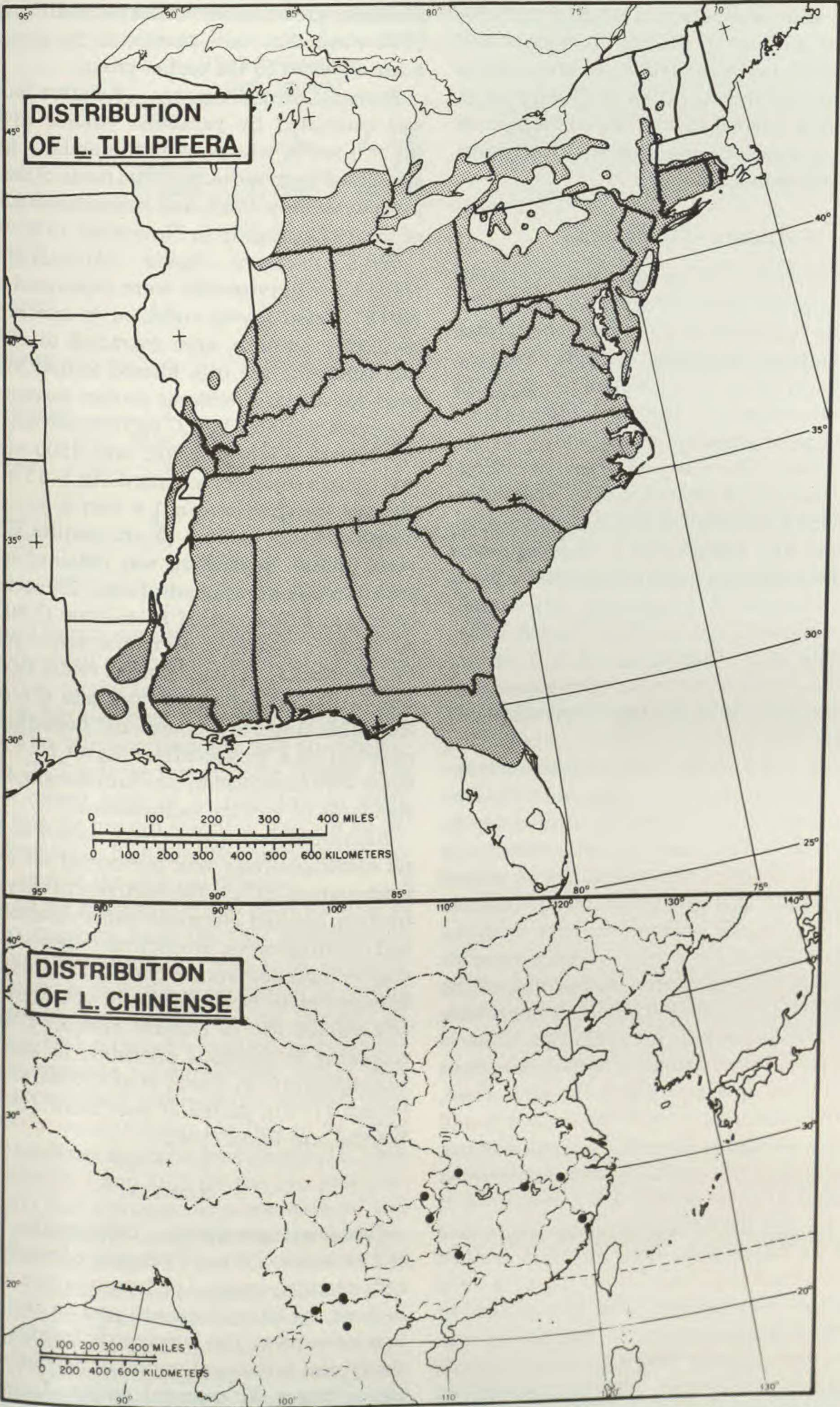


FIGURE 1. Distribution maps of *L. tulipifera* (Little, 1971) and *L. chinense* (Wu & Wang, 1957).



chemistry, and paleobotany. Our goal is to determine the amount of genetic divergence that has taken place in the genus *Liriodendron* and to gain insight into the evolution of divergence in a pair of long isolated species. In this report we will concern ourselves primarily with the results of the hybridization study.

#### MATERIALS AND METHODS

*Plant materials.* Seven accessions of *Liriodendron chinense* were available for this study. Six of these originated in 1948 from the Lushan Botanic Garden, Kuikiang, Kiangsi Province, China, by way of the Cabot Foundation and the Arnold Arboretum of Harvard University. Of these, five are maintained at the Blandy Experimental Farm, University of Virginia, Boyce, Virginia, and one is located in the Coker Arboretum on the University of North Carolina campus, Chapel Hill. The seventh *L. chinense* accession originated from a botanical garden in Hupei Province, China (A. R. Kruckeberg, pers. comm.) and was obtained from the MsK Nursery of Seattle, Washington. Individuals of *L. tulipifera* used in the crosses were native trees growing on or near the campus of the University of North Carolina.

*Crossing experiments and progeny maintenance.* Interspecific crosses between *Liriodendron tulipifera* and *L. chinense* were made in 1973 and 1977. The single tree of *L. chinense* in the Coker Arboretum was used as a male parent because it produced only a few, mostly inaccessible, flowers. Hand pollinations were made by applying pollen to the stigmas of buds emasculated one or two days prior to opening of the perianth. After pollen application, the buds were covered with paper bags for about two weeks to prevent pollen contamination. Self-pollinations were made by opening buds at the same stage, pollinating with pollen from the same tree and covering with bags as with the cross-pollinations. In the autumn fruits were harvested and stratified for at least 10 weeks at 4°C. They were sown in flats in the greenhouse, and seedlings were transplanted to 4-inch pots when they had reached the appropriate size. The saplings from the 1973 pollinations were planted in an alluvial field in the spring and summer of 1975. Individuals were spaced every six feet in rows ten feet apart. Weeds were partially controlled, but irrigation was not available. Seedlings from the 1977 group were treated initially as the earlier group, but were

container grown under uniform conditions until 1980 when they were planted in the nursery at a site adjacent to the earlier group.

*Biomass measurements.* Relative biomass was estimated by parabolic volume of boles ( $pv = 0.5\pi r^2 h$ , where  $r$  = radius and  $h$  = height) calculated from measurements made of the 1973 progeny in July 1982, and measurements made of the 1977 progeny in November 1979.

*High pressure liquid chromatography (HPLC).* Flavonoids were separated using HPLC. Dried leaves collected in late summer and early autumn were extracted in absolute methanol (0.5 g/5 ml), filtered to 0.45  $\mu$ m and used for analysis without further treatment. A Waters Associates HPLC (6000A solvent delivery system, U6K injector, and 4500 variable wavelength injector) was used. An 8–15  $\mu$ l sample was injected onto a 1.4 mm  $\times$  30 cm Ultrasphere-ODS column (5  $\mu$ m particle size, reverse phase). Separation was obtained using a mobile phase of tetrahydrofuran : 2% acetic acid (22:78) at a flow rate of 1.0 ml/min (2,800 psi), an isocratic modification of gradient techniques used by Asen (1977) and Smith (1980). Detection was accomplished at 340 nm and 0.1 AUFS. Retention times and compound quantities were recorded on a Perkin-Elmer Sigma 10. Components were identified by co-chromatography with standards or spectral examination.

*Electrophoresis and enzyme detection.* Starch gel electrophoresis was performed on extracts from mature leaves. The methods of enzyme extraction, gel and electrode buffer compositions, and electrophoresis procedures followed or were slightly modified from Wendel and Parks (1982). Subsequent to electrophoresis, gels were sliced and stained for 16 enzyme systems (Table 5), according to methods published in Baum and Scandellios (1979), Cardy et al. (1980), Shaw and Prasad (1970), Siciliano and Shaw (1976), or Wendel and Parks (1982).

#### RESULTS

*Crossing experiments.* Interspecific hybrids of *Liriodendron* showed vegetative heterosis from early seedling stages. The heterotic response was in both the rate of growth and in the size of vegetative parts. The interspecific hybrids showed the typical bronze coloration of new foliage that characterizes the paternal parent, *L. chinense*, but is never seen in *L. tulipifera*. Two-way chromatographic separations of flavonoid extractives



TABLE 1. Seed set and survival from hybridizations between *Liriodendron chinense* and *L. tulipifera*.

	1973 Pollinations (field grown)			1977 Pollinations (container grown)			
	Self- Polli- nations <i>L. tulip.</i>	<i>L. tulip.</i> × <i>L. tulip.</i>	<i>L. tulip.</i> × <i>L. chin.</i>	Self- Polli- nations <i>L. tulip.</i>	Open- Polli- nations <i>L. tulip.</i>	<i>L. tulip.</i> × <i>L. tulip.</i>	<i>L. tulip.</i> × <i>L. chin.</i>
Samaraceta harvested	5	7	15	11	38	14	12
Seeds germinated	11	127	273	19	138	231	230
Number germinated per samaracetum	2.2	18.1	18.2	1.7	3.6	16.5	19.2
Number surviving	Feb. 77 5	Feb. 77 64	Feb. 77 172	Nov. 79 8	Nov. 79 100	Nov. 79 132	Nov. 79 166
Percent survival	45	50	63	42	72	57	72

developed from a sample of hybrid trees showed essentially a full complementation of flavonoid extractives characteristic of the two parent species, as has also been reported by Santamour (1972). The heterotic response was so conspicuous by 1977 that we repeated the crosses with different parent trees of *L. tulipifera* to make certain that the growth response was not an artifact of unusually high specific combining ability of trees used in the original crosses.

Data on the germination and early survival of seedling progenies from the two hybridization series are summarized in Table 1. In *Liriodendron* samaras with fertile seeds cannot be readily distinguished from sterile samaras. Because all samaras of both types were planted, germination per fruit aggregate (samaracetum) rather than percent germination was recorded. In both years interspecific combinations resulted in as many or more seeds per aggregate germinated (18.2 and 19.2 versus 18.1 and 16.5 for the two years, respectively) than intraspecific combinations, and the percent survival was greater for the interspecific class of seedlings (63 and 72 versus 50 and 57). The number of viable seeds produced by the self-pollinations was very small. Most that germinated died in the seedling stages; however, two still survive from the first group of crosses. These are healthy trees only a little smaller than the average for trees of the same age derived from crosses between different *L. tulipifera* parents.

To add an additional comparison, open-pollinated fruits were collected from each of the parent trees used in the 1977 pollinations. Germination was very low (3.6 seedlings per aggregate) but the seedlings obtained showed good vigor and survivorship. Since some open-pollinated fruit aggregates produced several seedlings, whereas others produced few or none, we suspect that in the spring of 1977 inadequate cross-pollination was the rule for the *L. tulipifera* parents. In 1973 and 1977 open-pollinated fruits were collected from *L. chinense*, but a total of only three seedlings was obtained. [Open-pollinated seeds of *L. chinense* from Kuling, China had viability approximately equal to that of open-pollinated *L. tulipifera* in America (Johnson, 1948).] Since leaf extracts from the three open-pollinated *L. chinense* seedlings have flavonoid and isozyme profiles typical of the synthetic interspecific hybrids, *L. chinense* produced no seedlings through self-pollination.

As a measure of vegetative heterosis, biomass (bole volume) was calculated for both progenies (Tables 2 and 3). In all cases for the 1973 progeny, biomass was significantly greater ( $P < 0.05$ ) for interspecific trees than for trees resulting from intraspecific crosses. The 1977 crossing series was designed to test whether the heterosis observed in the first series could be repeated. Different *L. tulipifera* parent trees were used, and an attempt was made to equalize the number of crosses in



TABLE 2. 1982 biomass of *Liriodendron* progenies synthesized in 1973.

Cross	Number of Progeny	Biomass <sup>a</sup> in m <sup>3</sup>
<i>L. tulipifera</i> (Saunders tree <sup>b</sup> ) × <i>L. chinense</i>	46	0.108 <sup>c</sup>
<i>L. tulipifera</i> (Murphy tree <sup>b</sup> ) × <i>L. chinense</i>	93	0.095 <sup>c</sup>
<i>L. tulipifera</i> (Saunders tree) × <i>L. tulipifera</i> (Murphy tree)	53	0.074

<sup>a</sup> Biomass:  $PV = 0.5\pi r^2 h$ .  
<sup>b</sup> Large individual parent trees of *L. tulipifera* on the University of North Carolina campus.  
<sup>c</sup> Difference between interspecific and intraspecific hybrids significant at the 0.05 level.

all combinations. Nearly all pollinated flowers produced fruits, but many were destroyed by squirrels. Table 3 is a summary of measurements made of the products of this second series of crosses. The interspecific hybrids had significantly greater biomass ( $P < 0.001$ ) than the intraspecific hybrids or the open-pollinated group. The progeny of certain *L. tulipifera* parent trees consistently showed a greater heterotic response than others with respect to both inter- and intraspecific crosses, but this specific combining ability response did not obscure the rather great vegetative heterosis observed in the interspecific hybrids.

In the spring of 1981 several flowers were produced on a few of the interspecific and the intraspecific hybrid trees generated by the 1973 crosses. The flowers of the *Liriodendron tulipifera* × *L. chinense* hybrid were, like the leaves, somewhat larger than those of either parent species. Furthermore, orange pigment was present on most of the outer petal surface in flowers of the interspecific hybrid rather than being restricted to a narrow band toward the insertion of the petals as is typical of *L. tulipifera*. All available open-pollinated samaras from both the intra- and interspecific hybrid groups (a majority from the intraspecific hybrids) were planted in order to compare growth characteristics of the F<sub>2</sub> progenies. Four F<sub>2</sub> seedlings were obtained from the *L. tulipifera* × *L. chinense* hybrids, whereas about 40 seedlings were obtained from the hybrids between two *L. tulipifera* parents. The vigor and variability of both groups are about the same, and the F<sub>2</sub> seedlings from both groups appear indistinguishable.

TABLE 3. 1979 biomass of *Liriodendron* progenies synthesized in 1977.

Progeny	Number of Individuals	Biomass in cm <sup>3</sup>
<i>Liriodendron tulipifera</i> × <i>L. chinense</i> (3 different <i>L. tulipifera</i> parents)	164	19.23 <sup>a</sup>
<i>L. tulipifera</i> self-pollinations	10	5.38
<i>L. tulipifera</i> open-pollinations	103	13.10
<i>L. tulipifera</i> × <i>L. tulipifera</i> (4 trees involved in 5 different combinations)	134	9.25

<sup>a</sup> Significantly different from all other progenies at the 0.001 level.

*Flavonoid and enzyme analysis.* Leaf extracts from thirty populations (average of five trees each) of *Liriodendron tulipifera* and from the seven available trees of *L. chinense* were examined by high pressure liquid chromatography (HPLC). Fourteen probable, and four known, flavonoids (absorbent at 340 nm) from the *L. tulipifera* extracts were quantitatively assayed. All accessions of *L. tulipifera* were qualitatively alike in flavonoid makeup, but large quantitative differences were common. These quantitative differences are large enough to appear as qualitative differences on thin-layer or paper chromatographic separations of crude extracts. Small, but measurable quantitative differences were characteristic of wild trees from the same locality (population), while major quantitative differences often occurred between closely spaced populations. Inspection of the quantitative data (Table 4) for the 30 populations provided no obvious correlation with altitude or geographical location. Furthermore, principal component analysis of these data failed to reveal any obvious clustering of populations nor were there any significant correlations obtained between population positions along the first two principal components and altitude, latitude or longitude.

The flavonoid extractives from the five *Liriodendron chinense* trees from the Blandy Experimental Farm were very similar to each other, whereas the tree from the Coker Arboretum differed only slightly from them. In general the six trees are at least as similar to each other as trees



TABLE 4. Distribution of 340 nm absorbent extractives in 30 populations of *L. tulipifera*.

Popu- lation #	Location	Flavonoid and Flavonoid-like Extractives <sup>a</sup>																	
		1	2	3	4	5	6	7	8	9 <sup>b</sup>	10	11	12 <sup>c</sup>	13 <sup>d</sup>	14	15 <sup>e</sup>	16	17	18
1	Macon Co., NC	2.3	27.4	9.0	2.6	3.8	—	0.9	12.6	14.0	5.3	3.8	6.5	2.0	—	1.7	1.4	2.2	0.6
5	Rabun Co., GA	—	49.2	2.1	0.7	5.9	1.6	2.4	26.9	10.0	—	0.6	1.8	2.0	0.3	0.3	0.3	0.4	1.3
8	Transylvania Co., NC	—	32.3	11.0	4.5	4.7	3.2	2.9	0	6.8	0.5	1.5	4.4	2.2	—	0.2	1.3	4.0	0.9
10	Transylvania Co., NC	4.9	5.6	4.7	0.7	0.4	0.6	2.0	9.7	7.7	2.2	0.5	16.0	1.1	—	—	1.8	—	0.3
12	Macon Co., NC	—	36.4	0.2	1.9	3.8	0.7	0.9	28.0	12.2	2.0	1.6	3.7	1.8	0.2	0.5	0.4	0.7	1.1
16	Transylvania Co., NC	1.8	18.0	2.7	0.7	1.1	0.6	3.4	16.9	25.7	5.8	2.5	9.0	5.2	—	1.2	0.2	1.7	0.4
17	Henderson Co., NC	—	40.4	0.5	1.4	4.5	0.9	1.5	16.0	8.8	2.7	1.6	2.8	2.2	—	2.4	—	2.9	1.9
19	McDowell Co., NC	3.8	22.7	6.8	3.1	3.2	0.6	1.1	26.9	15.0	0.3	1.7	3.7	3.1	0.2	1.1	1.2	2.3	0.9
25	Swain Co., NC	—	31.4	10.1	3.9	5.3	0.9	0.3	1.0	15.9	4.1	3.0	4.5	2.0	0.3	0.9	1.1	1.5	0.9
28	Graham Co., NC	0.9	21.6	2.1	1.0	3.1	0.9	1.4	40.0	14.9	—	1.8	3.2	2.0	0.1	0.7	0.9	1.5	0.8
31	Graham Co., NC	—	25.2	3.4	2.4	2.3	0.4	0.2	27.8	12.2	2.8	1.7	3.5	2.7	0.5	0.8	0.7	1.1	0.8
32	Swain Co., NC	2.1	29.9	4.7	—	1.6	2.2	2.3	42.7	5.0	—	1.3	4.4	2.1	0.1	1.0	0.5	0.7	0.7
33	Macon Co., NC	5.8	34.0	—	0.1	3.7	2.5	2.8	25.8	10.0	0.3	1.5	2.1	1.9	0.1	0.4	0.4	1.7	0.6
41	Transylvania Co., NC	3.3	39.8	—	0.2	2.8	1.9	3.5	25.0	6.9	4.1	1.3	1.0	2.6	0.1	0.2	0.3	0.5	0.6
44	Macon Co., NC	—	38.3	9.6	2.5	3.8	2.5	2.4	4.0	21.4	—	1.0	3.8	2.6	0.1	—	0.8	2.4	1.8
46	Buncombe Co., NC	5.9	29.9	12.3	1.6	2.2	—	3.4	4.0	16.9	6.0	1.4	3.9	3.9	1.2	1.6	1.8	3.9	1.1
47	Buncombe Co., NC	4.7	26.7	4.5	2.2	3.2	1.5	2.5	16.0	16.0	2.4	0.8	4.0	3.5	0.1	0.7	—	1.2	0.7
55	Davidson Co., NC	—	49.9	—	3.4	13.3	2.0	2.7	0.3	2.1	1.1	—	2.4	2.2	—	—	—	—	2.6
57	Orange Co., NC	1.7	27.5	10.7	1.9	2.8	—	2.5	5.0	25.7	5.9	3.0	7.1	1.9	—	1.1	2.5	3.1	1.3
58	Stokes Co., NC	—	36.2	7.0	4.5	4.9	1.7	1.6	1.1	7.1	2.4	3.1	5.6	1.7	0.5	0.8	1.1	2.6	1.1
63	Jackson Co., NC	—	36.6	1.7	0.6	2.2	0.3	1.0	44.7	5.0	0.8	1.4	2.8	1.6	0.5	0.5	0.5	0.4	1.3
69	Avery Co., NC	4.3	28.9	9.4	4.0	2.2	1.0	1.2	4.0	16.8	3.1	3.0	4.9	2.8	0.8	1.1	1.6	2.9	1.1
71	Alleghany Co., NC	—	36.1	—	0.3	5.1	2.3	3.1	30.0	10.0	2.0	0.9	3.1	3.0	0.4	0.4	0.2	0.8	0.7
73	Floyd Co., VA	—	38.2	5.4	3.0	3.0	0.9	0.9	11.8	14.9	—	1.2	5.1	2.7	—	0.8	2.8	3.8	1.6
79	Greene Co., VA	3.5	36.2	7.0	1.2	8.2	—	2.0	3.0	15.0	4.3	0.8	4.6	3.0	—	2.1	1.3	3.6	1.9
83	Perry Co., PA	3.8	18.1	4.2	1.2	4.4	—	3.0	15.0	15.0	9.0	1.9	8.7	5.4	—	2.1	—	1.2	0.8
84	Snyder Co., PA	—	28.8	9.3	3.3	3.5	3.7	2.0	1.0	8.0	—	0.7	13.8	3.9	—	2.9	2.9	13.1	0.7
89	Lycoming Co., PA	—	35.3	10.5	4.3	3.8	1.6	2.6	12.0	10.0	—	0.5	1.5	2.9	0.1	2.5	2.6	4.4	1.7
82A	Watauga Co., NC	—	18.5	1.2	0.4	0.7	—	1.5	17.2	21.8	6.4	4.8	7.8	9.7	0.8	1.9	0.9	2.0	0.2
82B	Montgomery Co., VA	0.1	19.3	2.7	—	1.9	—	1.7	35.0	15.0	4.7	2.1	6.3	6.8	0.3	2.1	—	1.4	0.3

<sup>a</sup> Values indicate % of total extract.<sup>b</sup> Quercetin-3-rutinoside.<sup>c</sup> Kaempferol-3-rutinoside.<sup>d</sup> Quercetin-3-glucoside.<sup>e</sup> Kaempferol-3-glucoside.



TABLE 5. Enzymes resolved from *Liriodendron* leaves.

Alcohol dehydrogenase
Aldolase
Aspartate aminotransferase
Catalase
Diaphorase
Fluorescent esterase
Glutamate dehydrogenase
Glyceraldehyde-3-phosphate dehydrogenase
Isocitrate dehydrogenase
Malate dehydrogenase
Peroxidase
Phosphoglucose isomerase
Phosphoglucomutase
Superoxide dismutase
Shikimic acid dehydrogenase
Triose phosphate isomerase

of any single *L. tulipifera* stand. The seventh tree, from the MsK Nursery, is very different in its flavonoid pattern in comparison to the other six trees of *L. chinense*. The differences in flavonoid composition between the two accessions of *L. chinense* from divergent parts of China are at least as great as the maximum difference in flavonoids observed between different wild populations of *L. tulipifera*.

Sixteen enzyme systems were resolved in a survey of *Liriodendron* leaf extracts (Table 5). While it is uncertain how many genetic loci encode these systems, reasonable estimates can be obtained based upon the patterns of variation observed in the sampled material and upon a knowledge of the quaternary structure of each enzyme. Crossing experiments designed to document the genetic control of the resolved systems have been initiated, and the results of these experiments will be reported elsewhere (Wendel & Parks, unpub.). A preliminary assessment of allozyme variability revealed a sharp contrast in the degree of observed heterozygosity for the two species. While approximately 15 percent of the loci were heterozygous in a sample of three populations of *L. tulipifera*, not a single heterozygote has been observed in any of the seven *L. chinense* specimens examined. Six of these specimens (the Coker and the Blandy trees) were allozymically identical, whereas the seventh (the MsK tree) was very different. This last tree was apparently equally homozygous, but for alleles different than those of the other six trees at a number of loci.

## DISCUSSION

Despite a strong tendency for parthenocarpy (Stairs & Wilcox, 1966; pers. obs.), *Liriodendron* trees of either species set few viable samaras without cross-pollination; however, there is a low, but measurable, self-fertility in *L. tulipifera* (Table 1; Taft, 1966). It is thought that a large amount of self-pollination under natural conditions is the major cause of poor samara viability in *Liriodendron* (Guard, 1943; Santamour, 1972; Taft, 1966). In a number of studies (Guard, 1943; Santamour, 1972; Stairs & Wilcox, 1966; Taft, 1966) of *L. tulipifera*, controlled cross-pollination has very substantially increased the number of viable samaras per samaracetum. Similar mechanisms may be operating in *L. chinense*. Santamour (1972) recorded good seed set for *L. chinense* at the Blandy Experimental Farm, where tulip trees of both species are planted relatively close together. Our specimen of *L. chinense* at the Coker Arboretum, however, was isolated from all other individuals of *Liriodendron*, and we have obtained only three viable seeds from numerous samaraceta collected in two seasons.

Vegetative heterosis of the juvenile interspecific hybrid between *Liriodendron tulipifera* × *L. chinense* has also been observed in the United States by Santamour (1972) and in China by He (pers. comm.). The rapid growth rate has not diminished in trees that have reached reproductive age in any of the plantings. Intraspecific vegetative heterosis has been observed in *L. tulipifera* (Stairs, 1968; Stairs & Wilcox, 1966; Taft, 1966), but the increment of increase is not as great as that of the interspecific hybrid.

The production of vigorous  $F_1$  progenies from crosses between different species of the same genus of woody plants is not in itself unusual or highly significant. Grant (1971) named the kind of interspecific interfertility found in many woody plant genera the "*Ceanothus* pattern." This pattern is typified by *Magnolia*, in which there are few barriers to crosses between species of the same subgenus, despite their great geographical and temporal isolation, but a complete block to the successful hybridization of species belonging to the two different subgenera (Spongberg, 1976; Treseder, 1978). Because these hybrids have been made and used for horticultural purposes, there is little information about the fertility of the hybrids.

In woody plants successful crosses have been made, or reported, between various American,



European, or Asian species of *Abies* (Rohmeder, 1961), *Aesculus* (*A. × carnea* Hayne; Upcott, 1936), *Betula* (Johnson, 1939; Smith & Nichols, 1941), *Campsis* (*C. × tagliabuana* (Vis.) Rehder; Sax, 1933), *Castanea* (Johnson, 1939), *Catalpa* (*C. × hybrida* Hort. ex F. L. Späth; Smith, 1941), *Juglans* (Johnson, 1939), *Larix* (*L. eurolepis* Henry; Sax, 1932; Smith, 1941), *Liquidambar* (He & Santamour, pers. comm.), *Pinus* (Mirov, 1967), *Platanus* (*P. × acerifolia* (Ait.) Willd.; Sax, 1933), *Populus* (Johnson, 1939, 1942, 1946), *Quercus* (Johnson, 1939), and *Taxus* (Johnson, 1939).

Hybrid fertility and  $F_2$  vigor and variability are better indicators of species relationships in most woody plant genera than the mere fact of species compatibility. In five of these genera (*Campsis*, *Castanea*, *Catalpa*, *Platanus*, and *Populus*) the authors reported  $F_1$  fertility and the production of an  $F_2$  generation. Sax (1932) reported that the *Larix* hybrid had essentially normal cytological behavior in the  $F_1$ ; however, an  $F_2$  generation was not grown. The *Aesculus* hybrid, *A. × carnea*, was shown by Upcott (1936) to be a spontaneous allotetraploid with reduced  $F_1$  fertility.

In the other studies of hybridization of *Liriodendron tulipifera* and *L. chinense* (Santamour, 1972; and those made by Prof. Y. Peitzung at the Nanjing Botanical Garden in 1963 and 1965, *vide* correspondence with Prof. T. T. Yü, Academia Sinica, Beijing, China)  $F_2$  data have not yet been generated. Our results from the small  $F_2$  seed crop harvested in 1981 lead us to suspect that such hybrids will show considerable fertility and produce a relatively vigorous  $F_2$  population. The high level of heterosis observed in our *Liriodendron* hybrids coupled with the suggestion of hybrid fertility indicates that the Chinese and American species of *Liriodendron* have not diverged very far genetically despite long separation in time and space. This conclusion is currently being further evaluated: In the spring of 1982 a much larger number of flowers was produced on our  $F_1$  plants, and reciprocal sibling  $F_2$  crosses and backcrosses to *L. tulipifera* were made. (Unfortunately, no reproductive plants of *L. chinense* were available at that time.) Fruits from these crosses have been harvested, and a report on their germination and growth will appear at a later date.

In terms of their geographical ranges, the two species of *Liriodendron* cover similar total areas. However, *L. tulipifera* is a common and abun-

dant plant throughout much of the southeastern United States, whereas *L. chinense* is limited to a small number of individuals in several widely separated populations (Shan-an He, Jiangsu Institute of Botany, Nanjing, China, pers. comm.; Fig. 1). Individuals of *L. chinense* have a low degree of vigor (pers. comm.) which approximates that of our inbred *L. tulipifera* (one generation). Our very limited isozyme and flavonoid data on *L. chinense* indicate that these small populations have become genetically divergent, most likely as a result of drift. We suggest that the small number of extant trees of *L. chinense* suffer to some degree from inbreeding depression, a premise supported by the complete absence of isozyme heterozygosity. If this hypothesis is indeed true, hybrids between individuals from different populations should be markedly heterotic. It is our intention to expand the study of variability between and within *L. tulipifera* and *L. chinense* in collaboration with Chinese botanists.

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